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SHORT COMMUNICATION

FISH ANALYSIS OF A COMPLEX CHROMOSOME REARRANGEMENT INVOLVING NINE BREAKPOINTS ON CHROMOSOMES 6, 12, 14 AND 16

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SUMMARY

We report the prenatal diagnosis of an apparently balanced *de novo* complex chromosome rearrangement (CCR) which involved nine breakpoints on four different chromosomes. Fluorescence *in situ* hybridization (FISH) and spectral karyotyping (SKY) were performed as an adjunct to G-banding for characterization of the abnormal chromosomes. The 22-week female fetus showed minor dysmorphic features including dolichocephaly, broad fingernails, tibial bowing, clubfoot, thoracolumbar scoliosis and hypoplastic toenails. Autopsy revealed gall-bladder hypoplasia and an atrial septal defect. Chromosome analysis of fetal tissue confirmed the presence of the complex rearrangement. © 1998 John Wiley & Sons, Ltd.

KEY WORDS: complex chromosome rearrangement; prenatal diagnosis; fluorescence *in situ* hybridization (FISH); spectral karyotyping (SKY)

INTRODUCTION

Balanced reciprocal translocations between two chromosomes are relatively common, occurring in about 1 in 600 individuals. Complex rearrangements involving two or more chromosomes with three or more breakpoints occur much less frequently and may be balanced or unbalanced. Even when apparently balanced, CCRs are associated with a significant risk of mental retardation and phenotypic abnormalities. In fact, the most common means of ascertainment for CCRs is through an abnormal phenotype. Other indications for chromosome study leading to the detection of CCRs are recurrent pregnancy loss, previous abnormal abortus, infertility or subfertility, and prenatal diagnosis (Phelan *et al.*, 1990).

Only nine cases with apparently balanced CCRs have been found through prenatal diagnosis (Kohler *et al.*, 1986; Kim *et al.*, 1986; Bogart *et al.*, 1986; Bellec and de Perdigo, 1991; Batista *et al.*, 1993; Sikkema-Raddatz *et al.*, 1995; Delaroche *et al.*, 1995; Mercier *et al.*, 1996). Of these, seven rearrangements arose *de novo* and two were inherited from phenotypically normal mothers. We report the prenatal diagnosis of a *de novo* complex

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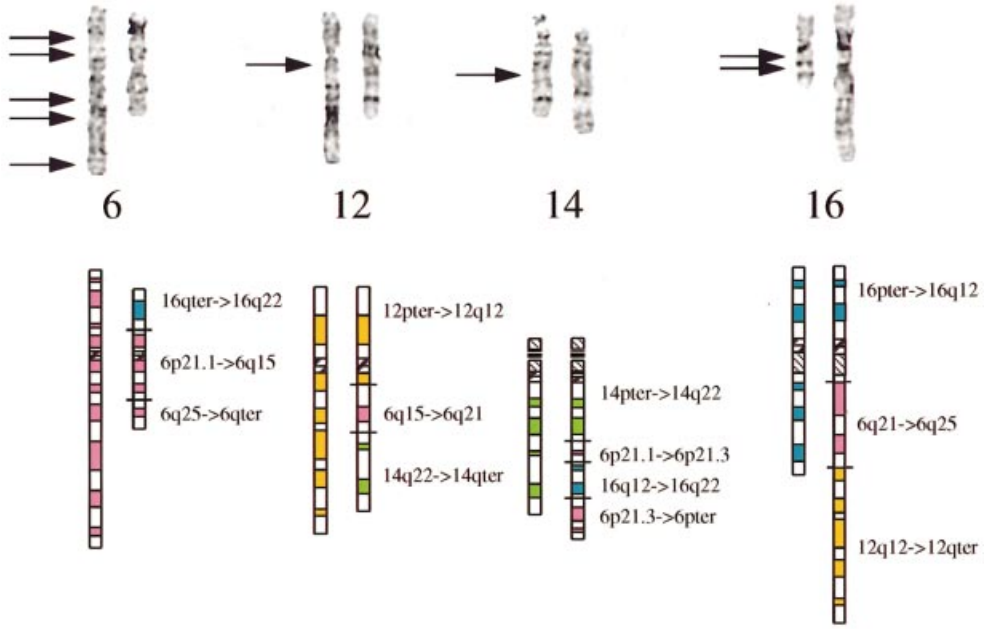

Fig. 1—Partial karyotype and idiogram of the complex chromosome rearrangement involving chromosomes 6, 12, 14 and 16

chromosome rearrangement involving four chromosomes and nine breakpoints. Fluorescence *in situ* hybridization (FISH) and spectral karyotyping (SKY) were used to characterize the abnormal chromosomes.

CASE REPORT

The mother was a 33-year-old, gravida 2, para 1, Japanese female referred for prenatal diagnosis due to an increased risk of trisomy 21. Maternal serum screening was performed at 17·6 weeks' gestation. When combined with maternal age, the triple-screen values indicated a 1 in 94 risk for trisomy 21. Amniocentesis was performed at 18 weeks' gestation and revealed a complex chromosome rearrangement involving chromosomes 6, 12, 14 and 16. The amniotic fluid alpha-fetoprotein level was 1·01 ug/ml (0·79 MoMs) and the acetylcholinesterase pattern was negative. Ultrasonogram obtained at the time of amniocentesis was interpreted as normal, with no evidence of fetal anomalies. Blood was obtained from both parents for chromosome analysis and revealed no evidence of a chromosome rearrangement. Family history was negative for birth defects or mental retardation and there was no history of preconceptual exposure to a chromosome breakage agent. The couple had a healthy two-year-old daughter.

The parents were referred for genetic counselling to discuss the risks associated with a *de novo* complex chromosome rearrangement. They were advised that *de novo* reciprocal translocations detected prenatally are typically associated with a 6 to 10 per cent risk for birth defects or significant learning problems. Taking into account the complex nature of the rearrangement detected in their fetus, they were advised that the risk may be as high as 50 per cent for physical or mental defects. Among the options discussed were a level II ultrasound examination to look closely for fetal defects, percutaneous umbilical blood sampling to confirm the presence of the complex rearrangement, and pregnancy termination. After careful consideration, the couple elected to terminate the pregnancy at 22 weeks of gestation.

FISH was performed to characterize the abnormal chromosomes. Whole chromosome 'paints' specific for chromosomes 6, 12, 14 and 16 were used (Oncor #P5210, #P5213, #P5215, #P5203). The results of G-banding and FISH suggested that there were a total of nine breakpoints: five on chromosome 6, one on chromosome 12, one on

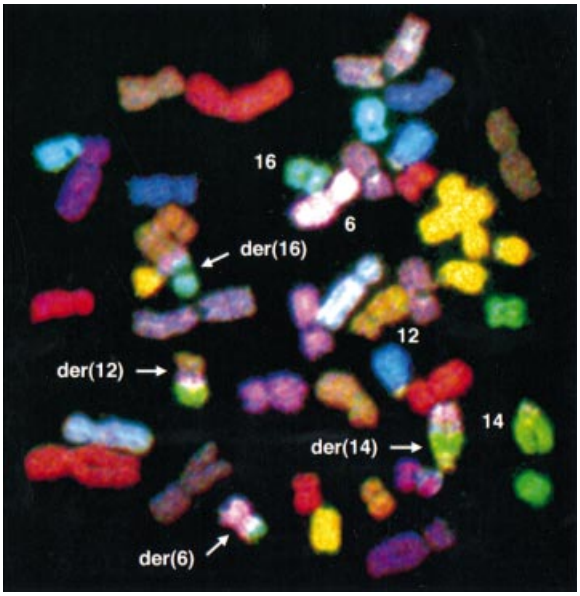

Fig. 2—SKY analysis showing the complex rearrangement in a single metaphase. The spread is shown by assigning red, green and blue colours to specific spectral ranges to convert the emission spectra of painting probes for visualization. The complex rearrangements are indicated by arrows. The result is consistent with FISH using individual painting probes

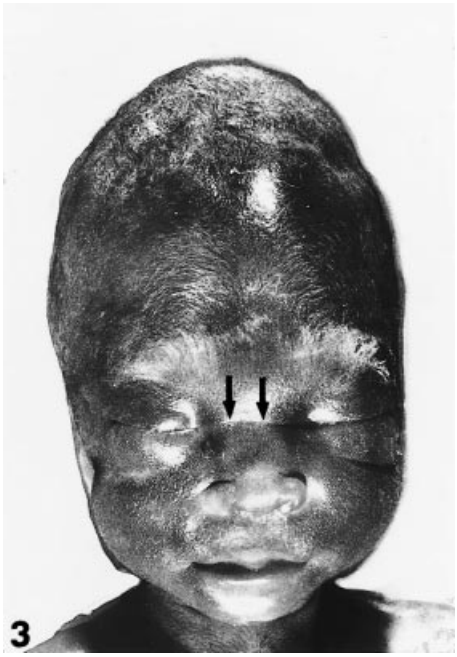

Fig. 3—Fetal face showing dolichocephaly, flat mid-face with horizontal nasal bridge skin crease (arrows), and long, flat philtrum

chromosome 14 and two on chromosome 16 (Fig. 1). The derivative chromosome 6 was composed of material from chromosomes 6 and 16. The distal long arm of 16 from 16qter to 16q22 was translocated to the short arm of the der(6) at 6p21.1. There were breaks at 6q15 and 6q25 with loss of the intervening segment.

The derivative 12 was composed of material from three chromosomes: 12, 6 and 14. Chromosome 12 was intact from 12pter to 12q12. An interstitial segment of chromosome 6 from 6q15 to 6q21 was translocated to 12q12. Distal to the segment from chromosome 6 was a segment of chromosome 14 from 14q22 to 14qter.

The derivative chromosome 14 was composed of the satellite, short arm and proximal long arm of 14, as well as a segment from chromosome 16 and two segments of chromosome 6. Chromosome 14 was intact from pter to 14q22. An interstitial segment of chromosome 6 from 6p21.1 to 6p21.3 was translocated to 14q22. A portion of chromosome 16 from 16q12 to 16q22 was translocated to 6p21.3. The segment 6p21.3 to 6pter was distal to the segment from chromosome 16.

The derivative chromosome 16 contained material from chromosomes 16, 6 and 12. There was a break at 16q12 with translocation of the segment 6q21 to 6q25. Distal to this segment was the long arm of chromosome 12 from 12q12 to 12qter.

The karyotype was designated 46,XX,der(6) t(6;16) (p21.1;q22)del(6) (q15q25), der(12) t(6;12) (q15; q12) t(6;14) (q21;q22), der(14) t(6;14) (p21.1; q22) ins(6;16) (p21.3;q12 q22), der(16) t(6;16) (q21;q12) t(6;12) (q25;q12).

Prepared microscope slides were referred for spectral karyotyping (SKY) to confirm the composition of the rearranged chromosomes (Fig. 2). Results of SKY coincided with the results obtained by FISH analysis using individual painting probes. Although the complex nature of the rearrangement was apparent by G-banding, the characterization of the abnormal chromosomes could not have been achieved without the use of FISH or SKY. In particular, the derivative chromosome 14 was interpreted by G-banding to consist of the satellites, short arm, centromere and proximal long arm of chromosome 14, a segment of the long arm of 16, and the distal short arm of chromosome 6. The fact that a segment of chromosome 6 was inserted between the segments from chromosomes 14 and 16 was not detected by G-banding, but was

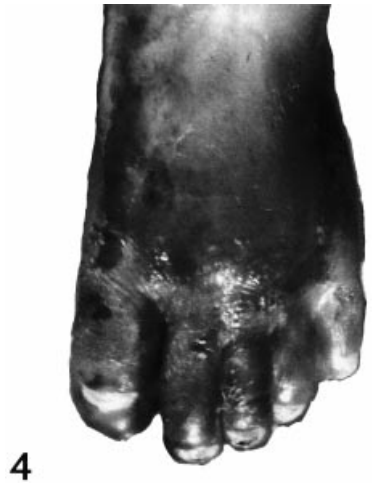

Fig. 4—Left, anterior foot showing short first toe with nail hypoplasia

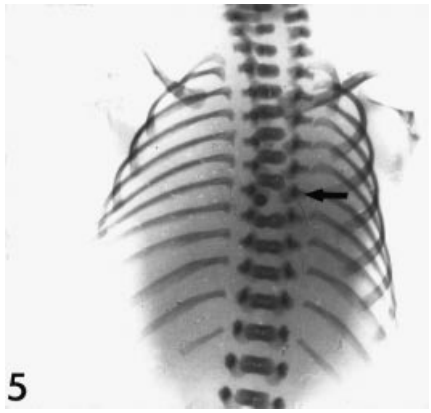

Fig. 5—Spinal X-ray showing hemivertebra at T-7 (arrow)

seen in both the FISH and the SKY preparations. The same karyotype interpretation was obtained by independent investigators using FISH (MCP and ECC) and SKY (ES, YN, TR), demonstrating that the techniques were equally effective in characterizing the rearrangements and that both of these molecular cytogenetic techniques were more effective than classical cytogenetics in resolving the complex karyotype.

Autopsy was performed on the 475 g female fetus (Fig. 3) and revealed several minor phenotypic aberrations including dolichocephaly, hirsute flat face, abnormal horizontal nasal bridge skin crease, flat philtrum, broad thumb nails, short great toes with hypoplastic nails (Fig. 4), clubfoot, mild thoraco-lumbar scoliosis and hemivertebra at T-7 (Fig. 5). Internal anomalies included atrial septal defect and hypoplastic gall-bladder (Fig. 6). Solid tissue obtained at autopsy (amnion, placental villi, gonad and kidney) showed the same complex chromosome rearrangement. No normal cells suggestive of a post-zygotic origin for the rearrangement were detected.

It was noted on G-banding and Q-banding that one maternal chromosome 14 had prominent satellites, as observed on the structurally normal 14 in the fetus. Neither of the paternal 14s had prominent satellites. The morphology of the satellite and stalk region on the derivative 14 in the fetus was consistent with the appearance of one chromosome 14 in the father. Chromosome polymorphisms were not informative in identifying the parental origin of the other derivative chromosomes. Nevertheless, if we assume that this was a pre-zygotic event, evidence suggests that the complex rearrangement occurred during paternal meiosis rather than maternal meiosis.

COMMENT

Complex chromosome rearrangements are considered uncommon, with about 100 such rearrangements reported (Mercier *et al.*, 1996). CCRs result when three or more independent breaks occur in two or more chromosomes and the broken segments rejoin at random to form various derivative chromosomes (Fuster *et al.*, 1997). Review of the literature reveals rearrangements involving from 2 to 7 chromosomes and from 3 to 10 breakpoints (Kousseff *et al.*, 1987; Tupler *et al.*, 1992). The event which initiates the chromosome breakage is unknown, although maternal exposure to potential mutagens has been implicated. Catti and Schmid (1971) reported a CCR in a child born to a mother who had occupational exposure to ionizing radiation. In 1977, Fitzgerald *et al.* (1977) reported a CCR in a child whose mother had untreated malignant melanoma; the authors suggested that a common unidentified agent may be involved in the mother's tumour and the child's chromosome abnormality. Ostrer *et al.* (1984) reported a *de novo* translocation and an unrelated deletion in a child whose mother had been treated with immunosuppressive agents for systemic lupus erythymatosus before and during pregnancy. Kousseff *et al.* (1987) suggested that maternal chromosome instability might have led to a CCR in a 12-year-old boy whose rearrangement involved seven chromosomes. Chromosome analysis of the mother revealed a

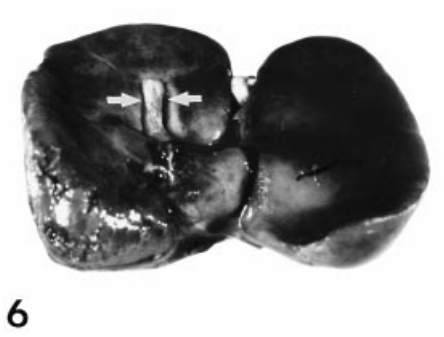

Fig. 6—Inferior view of liver showing hypoplastic gall-bladder (arrows)

low number of spreads with abnormal chromosomes, suggestive of chromosome instability. No predisposing factor leading to maternal chromosome instability was identified. In none of the above cases was the parental origin of the CCR determined, therefore the association with maternal exposure or maternal chromosome instability is purely speculative. In fact, the vast majority of *de novo* CCRs in which parental origin has been determined, have arisen in the father (Batista *et al.*, 1994).

In the case of Batista *et al.* (1993), the origin of one of four chromosomes involved in a CCR was determined to be paternal. Interestingly, the father worked in a chemical company, raising speculation about potential mutagen exposure. In the present case, the derivative chromosome 14 appears to be of paternal origin, but there is no history of paternal exposure to possible chromosome breakage agents.

Generally, the more chromosomes involved and the more breakpoints present, the more difficult it is to characterize the derivative chromosomes generated by the rearrangement. FISH has proven to be a useful adjunct to high resolution G-banded analysis in the elucidation of such rearrangements and in the detection of cryptic complex rearrangements that would be undetected by conventional cytogenetic methods (Batista *et al.*, 1993). In the present case, SKY (Schrock *et al.*, 1996, 1997) was also employed to permit accurate characterization of the derivative chromosomes. Results of the SKY analysis demonstrate the strength of this procedure in detecting the complexity of the rearrangement as well as the sensitivity of the procedure in discriminating between the various chromosome fluors on a single spread.

The present case represents the 10th reported case of an apparently balanced CCR detected prenatally. Table I lists the chromosomes involved in the rearrangements, the number of breakpoints, the indication for prenatal diagnosis, the presence or absence of fetal anomalies, and the outcome. The cases involve from two to four chromosomes with three to nine breakpoints. Only 2 of the 10 prenatally detected CCRs were inherited and both were transmitted from a carrier mother. This finding is consistent with reports in the literature that most CCRs arise *de novo*. Those rearrangements that are inherited are usually transmitted from a carrier mother while the *de novo* cases typically arise during spermatogenesis (Batista *et al.*, 1994). In our case the parental origin of only one of the derivative chromosomes was determined and the results were consistent with a paternal meiotic error. The low frequency of rearrangements transmitted through carrier males may reflect chromosome pairing problems during spermatogenesis manifested as infertility or subfertility in male carriers of CCRs (Batista *et al.*, 1994).

Two of the five cases in which the pregnancy was terminated showed no apparent anomalies at autopsy (Batista *et al.*, 1993; Sikkema-Raddatz *et al.*, 1995). In the case reported by Kim *et al.* (1986), the fetus had intra-uterine growth retardation, low-set ears, hypoplastic mandible and widely separated great toes. The case reported by Delaroche *et al.* (1995) had prenatal chromosome studies due to the finding of hypoplastic left heart on ultrasonography. Because the healthy mother carried the same chromosome rearrangement, the authors could not discern whether the heart defect was coincidental or resulted from the chromosome defect in the fetus. Our case had several minor dysmorphic features that may be attributable to the *de novo* rearrangement.

Of the five cases with postnatal follow-up, one with a maternally transmitted rearrangement was clinically normal at birth (Bellec and de Perdigo, 1991), two were apparently normal at two and three years of age (Sikkema-Raddatz *et al.*, 1995; Kohler *et al.*, 1986), one had growth and speech delay (Bogart *et al.*, 1986), and one had multiple congenital anomalies (Mercier *et al.*, 1996). The anomalies included poorly differentiated ears, short neck, widely spaced nipples, hypospadias, bilateral hydrocele, supernumerary distal forearm crease, bilateral single palmar crease, overlapping fingers and metatarsus valgus. Amniocentesis was performed after a routine ultrasonogram revealed

Table I—Description of *de novo* CCRs detected at prenatal diagnosis

Rearrangement	Number of breakpoints	Indication	Fetal anomalies	Outcome	Reference
t(6;12;14;16)dn	9	Risk tri 21	+	Terminated	Present case
t(2;3;4;13)dn	5	Abnormal US	+	MCA	Mercier *et al.* (1996)
t(2;21;18)mat	3	Hypoplastic left heart	+	Terminated	Delaroche *et al.* (1995)
t(3;4;10;17)dn	7	AMA	−	Terminated	Sikkema-Raddatz *et al.* (1995)
t(2;5;18)dn	5	AMA	−	Normal at 3 years	Sikkema-Raddatz *et al.* (1995)
t(1;3;9)dn	9	Low MSAFP	−	Terminated	Batista *et al.* (1993)
t(2;7;10)mat	?	?	−	Normal at birth	Bellec and de Perdigo (1991)
t(7;7;14)dn	3	AMA	−	Normal at 2 years	Kohler *et al.* (1986)
t(6;11;21)dn	4	FH NTD	−	Growth delay	Bogart *et al.* (1986)
t(4;15;16;6)dn	4	FH MR	+	Terminated	Kim *et al.* (1986)

US: ultrasonogram; AMA: advanced maternal age; MSAFP: maternal serum alpha-fetoprotein; FH: family history; NTD: neural tube defect; MR: mental retardation.

slight hydramnios and hands that were always held in a closed position. The infant was diagnosed during the neonatal period with Hirschsprung disease and his rearrangement involved a breakpoint at 13q34, a region previously implicated in Hirschsprung disease (Mercier *et al.*, 1996). A recessive gene for Hirschsprung disease has in fact been mapped to 13q22 (Puffenberger *et al.*, 1994). The patient reported by Bogart *et al.* (1986) was observed to be normal at birth but showed delay in both growth and speech at $2\frac{1}{2}$ years of age, thus demonstrating the need for long-term follow-up in assessing the consequences of apparently balanced CCRs.

For simple *de novo* translocations detected at prenatal diagnosis, Warburton (1991) found the risk of malformations and mental retardation to be about 6·1 per cent. The risk associated with *de novo* CCRs is expected to be higher than the risk associated with simple translocations, since the number of chromosomes involved and the number of breakpoints is greater in CCRs. As the complexity of the rearrangement increases, the characterization of the derivative chromosomes and the accurate definition of breakpoints become more difficult. The ability to detect minute deletions or duplications is compromised as the ability to define the breakpoints is impaired. Also, as more breakpoints are involved, more likely is a gene disruptive event which could lead to phenotypic abnormalities (Batista *et al.*, 1994). While molecular cytogenetic techniques, such as FISH and SKY, have improved the ability to characterize CCRs, they do not permit the detection of submicroscopic deletions or duplications, gene disruption, or gene position effect. All of these have been invoked to explain the abnormal phenotypes in carriers of apparently balanced rearrangements (Mercier *et al.*, 1996).

Among prenatally diagnosed CCRs, 2 of 10 cases had anomalies detected by prenatal ultrasonogram. Of the remaining eight cases, two had fetal anomalies and one had growth deficiency and developmental delay. Taking into account that the cases described as phenotypically normal included two fetuses, a newborn, a two-year-old and a three-year-old, it is apparent that long-term follow-up data on prenatally diagnosed CCRs are lacking and that the risk of 50 per cent for structural malformations or mental retardation is probably an underestimate.

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